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HLA-DQA1 & DQB1 variants associated with hepatitis B virus-related chronic hepatitis, cirrhosis & hepatocellular carcinoma

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***Background & objectives*: Clinical outcome after hepatitis B virus (HBV) exposure varies extremely from spontaneous clearance to chronic hepatitis B and often progresses to liver cirrhosis (LC) and hepatocellular carcinoma (HCC). Host genetic factor plays an important role in the regulation of immune response. This study was aimed to investigate whether HLA class II DQA1 and DQB1 gene polymorphism were associated with chronic hepatitis B infection and in the development of HBV-related LC and HCC.**

***Methods*: DQA1 and DQB1 allele polymorphism were studied in 187 patients with HBV-related liver diseases (which included 73 chronic hepatitis B, 84 LC and 30 HCC patients) and 109 controls who had spontaneously recovered from HBV infection using polymerase chain reaction amplification with sequence-specific primers.**

***Results*: Our data suggested that DQA1*0101/2/4 [odds ratio (OR)=2.78; P^c=0.003], DQA1*0103 (OR=2.64; P^c=0.0007) and DQB1*0302/3 (OR=2.15; P^c=0.01) were associated with the protection from chronic HBV infection, whereas DQB1*0402 (OR=0.25; P^c=0.001) showed susceptible effect on chronic HBV infection. DQB1*0601 (OR=3.73; P^c=0.006) conferred protective effect from developing LC; similarly, DQB1*0302/3 (OR=5.53; P^c=0.05) and DQB1*0402 (OR=0.00; P^c=0.001) conferred protective effect from developing HCC. However, DQA1*0601 and DQB1*0503 showed susceptible effect on chronic HBV infection; these associations were no longer significant after Bonferroni correction.**

***Interpretation & conclusions*: Our results revealed HLA-DQA1*0101/2/4 - DQA1*0103 - DQB1*0302/3 and DQB1*0601 as protective and DQB1*0402 as risk alleles. The study suggests that various subtypes of HLA-DQA1 and DQB1 are associated with both HBV clearance and development of chronic HBV infections.**

Key words Chronic hepatitis B - hepatitis B virus - hepatocellular carcinoma - human leucocyte antigen - liver cirrhosis - polymorphism

Hepatitis B virus (HBV) is one of the most common causes of liver diseases including cirrhosis and hepatocellular carcinoma (HCC)[1]. During the lifetime, despite the fact that only 2-10 per cent of HBV-infected individuals develop chronic complications of liver disease, the clinical outcomes differ with 15-40 per cent

higher risk of developing liver cirrhosis (LC) and HCC[2]. Primary HBV infection in the host may be either symptomatic or asymptomatic, where young children are mostly asymptomatic and adults self-limited, with viral clearance and development of lasting immunity to reinfection[3]. Some primary HBV infections in adults (<5%) do not resolve and develop into persistent infections[3]. Among the chronic hepatitis B, 20 per cent develop LC (a condition where regenerative nodules and fibrosis coexist with severe liver injury) and less than five per cent develop HCC with long-term disease progression. However, the factors determining the chronicity of primary HBV infection and its disease progression to LC and HCC are not clearly understood.

It has been shown that outcome of the HBV infection is not because of the variation in the virulence of viral strains[4], it may rather be because of host-immune response[5]. Furthermore, a study conducted in HBV infected twins in China showed a strong genetic association with the outcome of HBV infection and indicated that host genetic factors might play a role in influencing the clinical phenotype[6]. First-degree relatives of HBV-related HCC patients were supposed to be at increased risk of HCC[7]. Thus, there was a probability that immunogenetic factors might influence disease severity and different clinical outcomes.

Several studies have shown association of HLA class II genes with the outcome of HBV infections[8-12]. However, mostly, the results were inconsistent due to disparities in the study design, sample size and ethnicity. Many researchers have investigated the association of HLA genes with HBV-related HCC[13-17]. There is a paucity of Indian HLA studies in these patients. Therefore, the current study was undertaken to investigate the HLA allele polymorphism associated with HBV infection and disease progression to LC and HCC. The study was designed to characterize HLA-DQA1 and DQB1 gene polymorphism in chronic HBV infection and their possible association with the development of HBV-related LC and HCC in Indian patients.

Material & Methods

Patients were selected from the outpatient department of Medicine, Lok Nayak Jai Prakash Narayan Hospital, New Delhi, India, from February 2010 to February 2013. The sample size was calculated by using the online sample size calculator, Raosoft (*http://www.raosoft.com/samplesize.html*). A total of 296 HBV cases were genotyped and analyzed in this study. The patients were categorized into two groups, 187 HBV-related liver disease (Group I) with persistent hepatitis B surface antigen (HBsAg) positivity for more than six months including 73 chronic hepatitis B [male/female = 53/20; mean age = 29.0±13.3 yr; hepatitis B e antigen (HBeAg) positive 39], 84 LC (male/female = 69/15; mean age = 40.8±14.6 yr; HBeAg positive 57) and 30 HCC (male/female = 27/3; mean age = 55.2±11.7 yr; HBeAg positive 11) and 109 individuals who have spontaneously recovered (male/female = 82/27; mean age = 29.8±11.7 yr) from HBV infection serve as controls (Group II) (Table I). Informed written consent was taken from all the patients before enrolling in the study.

The following demographic and clinical parameters were recorded at the time of peripheral blood collection: gender, age, serum aspartate aminotransferase (AST) levels, alanine aminotransferase (ALT) levels, alkaline phosphatase levels and serological HBV markers. Individuals who were found to be positive for HBsAg for more than six months, with persistent or intermittent elevation in ALT/AST levels, HBV DNA viral load $>10^5$ copies/ml and liver biopsy showing chronic hepatitis (necroinflammatory score ≥4) were diagnosed as chronic hepatitis B as per the American Association for the Study of Liver Diseases (AASLD) guidelines[18],

Table I. Baseline clinical, demographical and laboratory characteristics of hepatitis B virus-infected patients

Patient group	No. of patients	Gender (male/female)	Age (yr)	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	HBeAg (%)
Spontaneously recovered	109	82/27	29.8±11.7	35.6±16.4	46.8±27.0	160.4±69.0	0/109 (0)
Chronic hepatitis B	73	53/20	29.0±13.3	98.7±244.5	120.4±302.2	218.9±100.1	39/73 (53.4)
Liver cirrhosis	84	69/15	40.8±14.6	81.9±51.2	65.0±41.8	169.4±72.3	57/84 (67.8)
HCC	30	27/3	55.2±11.7	73.3±47.5	109±105.2	228.7±183.2	11/30 (36.6)

Values are mean±SD. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; HCC, Hepatocellular carcinoma; HBeAg, hepatitis B e antigen

while the spontaneously recovered group was identified as individuals positive for anti-HBs and anti-HBc antibody and negative for HBsAg and HBeAg. LC was diagnosed on the basis of showing the presence of oesophagal varices by endoscopic examination, and clinical and biochemical/radiological evidence showing small and nodular liver in advanced cirrhosis along with increased echogenicity and irregular appearing areas. HCC was diagnosed as per the European Association for the Study of the Liver (EASL) diagnostic criteria[19] based on either fine needle aspiration cytology or by arterialization on colour Doppler, magnetic resonance imaging and alpha-foetoprotein >400 ng/ml. The study was approved by the Institutional Ethical Committee of Maulana Azad Medical College, New Delhi.

Serological testing: Blood samples (5 ml) were collected from all the participants and serum separated and stored at 20°C. Enzyme-linked immunosorbent assay was used to detect serum HBsAg, anti-HBc, anti-HBs and HBeAg according to the manufacturer's instruction (Radim Diagnostics, Italy).

DNA extraction: Genomic DNA was extracted from peripheral blood cells using QIAamp Blood Kit (Qiagen, Germantown, USA). All DNA samples were diluted to 100 ng/µl and stored at 4°C in Tris-EDTA buffer before use.

HLA-DQA1 and DQB1 allele polymorphism: HLA-DQA1 and DQB1 polymorphisms were detected by the sequence-specific primer (SSP)-PCR designed by Olerup *et al*[20] and synthesized by Sigma-Aldrich, USA. In each PCR, an internal positive control was included that amplified human growth hormone gene. The 5'-primer (5'TGC CAA GTG GAG CAC CCA A3') and 3'-primer (5'GCA TCT TGC TCT GTG CAG AT3') gave rise to 796 base pair (bp) fragment. A total amount of 25 µl PCR reaction mixture was prepared which contained 1 µl of genomic DNA (100 ng/µl), 2.5 µl of 10× reaction buffer (1% Triton X-100, 500 mm KCl, 100 mm Tris-HCl, *p*H 9.0), 2 µl of $MgCl_2$ (25 mmol/l), 0.25 µl of dNTP (10 mmol/l), 1 µl of each SSP (5 µmol/l), 0.2 µl of the internal positive control each (5 µmol/l), 0.1 µl of Taq polymerase (5 U/µl) and 16.75 µl of molecular grade water. PCR amplification was carried out in Biometra T-Gradient Thermal Cycler (Goettingen Germany). The PCR cycling conditions for HLA-DQA1 and DQB1 alleles were as follows: predenaturation at 94°C for 4 min, denaturation at 94°C for 60 sec, annealing at 65°C for 60 sec, extension at 72°C for 60 sec, repetition for 35 cycles and final extension at 72°C for five minutes. PCR products were electrophoresed in 2 per cent agarose gels stained with ethidium bromide. The gels were examined under ultraviolet transillumination (Vilber Lourmat Gel documentation system) and documented. PCR results were interpreted based on the presence or absence of specific amplified product with specific bp size and with the presence of internal amplification control.

Statistical analysis: HLA-DQA1 and DQB1 alleles were calculated by direct count. The phenotypic frequencies of the alleles were compared between HBV carriers and spontaneously recovered groups using Chi-square test or Fisher's exact test when required. A large number of alleles were tested, so there may be a chance of significant association when using multiple comparisons. Those *P* values were corrected by Bonferroni correction (P^c=P×the number of alleles observed)[21]. Odds ratios (OR) with 95 per cent confidence intervals were calculated.

Results

Comparison of frequency of HLA-DQA1 and DQB1 allele between groups I and II: HLA-DQA1*0101/2/4 showed a significant difference between spontaneously recovered and persistent HBV infection. The frequency of individuals carrying the DQA1*0101/2/4 allele was 66.0 per cent among spontaneously recovered, whereas it was only 41.1 per cent among persistent infections (χ^2=17.05, P=0.00003, P^c=0.003, OR=2.78) suggesting that this allele was protective against HBV infection. Similarly, DQA1*0103 was significantly increased in spontaneously recovered individuals compared to those with persistent infections (59.6 vs. 35.8%, χ^2=15.79, P=0.00007, P^c=0.0007, OR=2.64) indicating protective effect of this allele against HBV infection. DQA1*0104 and DQA1*0601 showed significant difference between spontaneously recovered and persistent infection (71.5 vs. 57.7%, χ^2=5.62, P=0.017, P^c=0.18, OR=1.84 and 18.3 vs. 9.6%, χ^2=4.68, P=0.030, P^c=0.33, OR=2.11, respectively), showing some protective effects against HBV infection. However, after Bonferroni correction, this association was no longer found to be significant (Table II).

Phenotypic frequency of HLA-DQB1 alleles is shown in Table III. In spontaneously recovered individuals, the frequencies of DQB1*0302/3 were significantly increased compared with the persistent infection (56.8 vs 37.9%, χ^2=9.95 P=0.001,

Table II. Frequency of HLA-DQA1 alleles in hepatitis B virus infections and control group

DQA1	Spontaneously recovered (n=109), n (%)	Persistent HBV infection (n=187), n (%)	χ^2	*P*	P^c	OR (95% CI)
DQA1*0101/4	13 (1.2)	22 (11.7)	0.021	0.884	1	1.016 (0.489-2.108)
DQA1*0101/2/4	72 (66.0)	77 (41.1)	17.05	0.00003	0.003	2.78 (1.7-4.546)
DQA1*0102/3	43 (39.4)	82 (43.8)	0.546	0.459	1	0.834 (0.515-1.349)
DQA1*0103	65 (59.6)	67 (35.8)	15.79	0.00007	0.0007	2.646 (1.628-4.3)
DQA1*0104	78 (71.5)	108 (57.7)	5.62	0.017	0.18	1.841 (1.108-3.057)
DQA1*0301	13 (11.9)	11 (5.8)	3.376	0.066	1	2.167 (0.934-5.021)
DQA1*0401	82 (75.2)	140 (74.8)	0.004	0.944	1	1.02 (0.590-1.76)
DQA1*0501	42 (38.5)	67 (35.8)	0.216	0.641	1	1.123 (0.689-1.829)
DQA1*0601	20 (18.3)	18 (9.6)	4.683	0.030	0.33	2.11 (1.062-4.192)
DQA1*0201	18 (16.5)	20 (10.6)	2.083	0.149	1	1.652 (0.831-3.28)
DQA1*0302	4 (3.6)	6 (3.2)	0.044	0.832	1	1.149 (0.317-4.165)

χ^2/Fisher's exact test. P^c, corrected *P* value after applying Bonferroni's correction; OR, odds ratio; CI, confidence interval; HBV, hepatitis B virus

Table III. Frequency of HLA-DQB1 alleles in hepatitis B virus infections and control group

DQB1	Spontaneously recovered (n=109), n (%)	Persistent HBV infection (n=187), n (%)	χ^2	*P*	P^c	OR (95% CI)
DQB1*0201	42 (38.5)	70 (37.4)	0.035	0.850	1	1.048 (0.644-1.704)
DQB1*0301/4	30 (27.5)	47 (25.1)	0.204	0.651	1	1.131 (0.662-1.931)
DQB1*0302/3	62 (56.8)	71 (37.9)	9.955	0.001	0.013	2.155 (1.333-3.485)
DQB1*0303	16 (14.6)	24 (12.8)	0.200	0.654	1	1.168 (0.590-2.311)
DQB1*0401	0	3 (1.6)	1.767	0.184	1	0.000 (0.000-0.000)
DQB1*0402	10 (9.1)	53 (27.8)	15.1	0.0001	0.0013	0.255 (0.123-0.526)
DQB1*0501	4 (3.6)	1 (0.5)	4.075	0.043	0.55	7.086 (0.781-64.22)
DQB1*0503	18 (16.5)	28 (14.9)	0.124	0.724	1	1.123 (0.588-2.142)
DQB1*0601	32 (29.3)	42 (22.4)	1.747	0.186	1	1.435 (0.839-2.454)
DQB1*0602	9 (8.2)	3 (1.6)	7.835	0.005	0.06	5.52 (1.461-20.85)
DQB1*0502	13 (11.9)	22 (11.7)	0.021	0.884	1	1.016 (0.484-2.108)
DQB1*0603	5 (4.5)	8 (4.2)	0.015	0.900	1	1.076 (0.343-3.374)
DQB1*0604	8 (7.3)	4 (2.1)	4.788	0.028	0.36	3.624 (1.065-12.33)

χ^2/Fisher's exact test. P^c, corrected *P* value after applying Bonferroni's correction

P^c=0.013, OR=2.15). Conversely, DQB1*0402 was significantly increased in persistent HBV infection compared with spontaneously recovered (27.8 vs 9.1%, χ^2=15.1, *P*=0.0001, P^c=0.001, OR=0.25). This difference was significant after Bonferroni correction also, which suggested a susceptible effect on HBV infection. DQB1*0501, DQB1*0602 and DQB1*0604 showed significant difference between spontaneously recovered and persistent infection (3.6 vs. 0.5%, χ^2=4.07, *P*=0.04, P^c=0.55, OR=7.08; 8.2 vs. 1.6%, χ^2=7.83, *P*=0.005, P^c=0.06, OR=5.52 and 7.3 vs. 2.1%, χ^2=4.78, *P*=0.02, P^c=0.36, OR=3.62, respectively), showing some protective effects against HBV infection. However, after Bonferroni correction, this association was no longer found to be significant.

Comparison of phenotypic frequency of HLA-DQA1 and DQB1 alleles in persistent HBV infection with and without liver cirrhosis: The phenotype frequency of DQA1*0601 was significantly increased in persistent infection without LC (13.6%) compared to those with LC (3.5%) suggesting a protective effect of this allele against the development of LC. However,

this difference was not significant after Bonferroni correction (χ^2=5.27, *P*=0.02, P^c=0.23, OR=4.28). No other HLA-DQA1 alleles showed association (Table IV). DQB1*0601 was significantly increased in persistent infection without LC (38.5%) compared to those with LC (14.2%), which suggested protective effect of this allele against the development of LC. This difference was significant after Bonferroni correction also (χ^2=11.92, *P*=0.0005, P^c=0.006, OR=3.73). Similarly, DQB1*0503 showed significant difference between persistent infection without LC and with LC (21.9 vs. 11.9%, χ^2=2.83, *P*=0.04, P^c=0.59, OR=2.07) showing protective effect of this allele against the development of LC. However, this difference was not significant after Bonferroni correction (Table V).

Comparison of phenotypic frequency of HLA-DQA1 and DQB1 alleles in persistent HBV infection with and without HCC: DQA1*0601 was significantly increased in HBV infection with HCC (16.6%) compared to those without HCC (3.5%), which suggested susceptible effect of this allele in the development of HCC (χ^2=5.80, *P*=0.015, P^c=0.16, OR=0.18). However, this difference was not significant after Bonferroni correction (Table VI). Conversely, DQB1*0302/3 and DQB1*0402 were significantly increased in patients

Table IV. Frequency of HLA-DQA1 alleles in hepatitis B virus infection with or without liver cirrhosis

DQA1	Without LC (n=73), n (%)	With LC (n=84), n (%)	χ^2	*P*	P^c	OR (95% CI)
DQA1*0101/4	8 (10.9)	12 (14.2)	0.388	0.532	1	0.738 (0.284-1.92)
DQA1*0101/2/4	31 (42.4)	36 (42.8)	0.012	0.910	1	0.984 (0.522-1.855)
DQA1*0102/3	37 (50.6)	34 (40.4)	1.643	0.200	1	1.511 (0.802-2.846)
DQA1*0103	27 (36.9)	27 (32.1)	0.406	0.524	1	1.239 (0.640-2.397)
DQA1*0104	43 (58.9)	48 (57.1)	0.049	0.823	1	1.075 (0.569-2.03)
DQA1*0301	4 (5.4)	7 (8.3)	0.488	0.484	1	0.637 (0.179-2.272)
DQA1*0401	55 (75.3)	62 (73.8)	0.048	0.826	1	1.084 (0.527-2.23)
DQA1*0501	31 (42.4)	28 (33.3)	1.389	0.239	1	1.476 (0.771-2.825)
DQA1*0601	10 (13.6)	3 (3.5)	5.274	0.021	0.23	4.286 (1.132-16.23)
DQA1*0201	7 (9.5)	12 (14.2)	0.809	0.368	1	0.636 (0.236-1.713)
DQA1*0302	3 (4.1)	3 (3.5)	0.030	0.860	1	1.157 (0.226-5.917)

χ^2/Fisher's exact test. P^c, corrected *P* value after applying Bonferroni's correction
LC, liver cirrhosis

Table V. Frequency of HLA-DQB1 alleles in hepatitis B virus infection with or without liver cirrhosis

DQB1	Without LC (n=73), n (%)	With LC (n=84), n (%)	χ^2	*P*	P^c	OR (95% CI)
DQB1*0201	27 (36.9)	36 (42.8)	0.560	0.454	1	0.782 (0.411-1.488)
DQB1*0301/4	24 (32.8)	18 (21.4)	2.612	0.106	1	1.796 (0.879-3.668)
DQB1*0302/3	36 (49.3)	32 (38.0)	2.003	0.157	1	1.581 (0.877-2.986)
DQB1*0303	11 (15.0)	12 (14.2)	0.019	0.890	1	1.065 (0.439-2.581)
DQB1*0401	1 (1.3)	2 (2.3)	0.213	0.644	1	0.569 (0.050-6.411)
DQB1*0402	22 (30.1)	30 (35.7)	0.464	0.495	1	0.792 (0.404-1.55)
DQB1*0501	0 (0)	1 (1.1)	0.874	0.349	1	0.000 (0.000-0.000)
DQB1*0503	16 (21.9)	10 (11.9)	2.834	0.046	0.59	2.077 (0.877-4.92)
DQB1*0601	28 (38.5)	12 (14.2)	11.92	0.0005	0.006	3.733 (1.725-8.079)
DQB1*0602	0 (0)	2 (2.3)	1.761	0.184	1	0.000 (0.000-0.000)
DQB1*0502	9 (12.3)	11 (13.0)	0.020	0.885	1	0.933 (0.363-2.395)
DQB1*0603	3 (4.1)	5 (5.9)	0.274	0.600	1	0.677 (0.156-2.936)
DQB1*0604	2 (2.7)	2 (2.3)	0.020	0.886	1	1.155 (0.158-8.41)

χ^2/Fisher's exact test. P^c, corrected *P* value after applying Bonferroni's correction

Table VI. Frequency of HLA-DQA1 alleles in hepatitis B virus infection with or without hepatocellular carcinoma (HCC)

DQA1	Without HCC (n=84), n (%)	With HCC (n=30), n (%)	χ^2	*P*	P^c	OR (95% CI)
DQA1*0101/4	12 (14.2)	2 (6.6)	1.191	0.276	1	2.333 (0.490-11.1)
DQA1*0101/2/4	36 (42.8)	10 (33.3)	0.833	0.361	1	1.5 (0.626-3.593)
DQA1*0102/3	34 (40.4)	11 (36.6)	0.134	0.714	1	1.175 (0.496-2.778)
DQA1*0103	27 (32.1)	13 (43.3)	1.215	0.271	1	0.619 (0.263-1.457)
DQA1*0104	48 (57.1)	17 (56.6)	0.028	0.865	1	1.02 (0.439-2.365)
DQA1*0301	7 (8.3)	0	2.664	0.102	1	0.000 (0.000-0.000)
DQA1*0401	62 (73.8)	23 (76.6)	0.095	0.757	1	0.857 (0.323-2.276)
DQA1*0501	28 (33.3)	8 (26.6)	0.454	0.500	1	1.375 (0.543-3.477)
DQA1*0601	3 (3.5)	5 (16.6)	5.809	0.015	0.16	0.185 (0.041-0.829)
DQA1*0201	12 (14.2)	1 (3.3)	2.625	0.105	1	4.833 (0.600-38.88)
DQA1*0302	3 (3.5)	0	1.1	0.295	1	0.000 (0.000-0.000)

χ^2/Fisher's exact test. P^c, corrected *P* value after applying Bonferroni's correction

Table VII. Frequency of HLA-DQB1 alleles in hepatitis B virus infection with or without hepatocellular carcinoma

DQB1	Without HCC (n=84), n (%)	With HCC (n=30), n (%)	χ^2	*P*	P^c	OR (95% CI)
DQB1*0201	36 (42.8)	7 (23.3)	3.587	0.029	1	2.464 (0.953-6.372)
DQB1*0301/4	18 (21.4)	5 (16.6)	0.311	0.576	1	1.364 (0.457-4.066)
DQB1*0302/3	32 (38.0)	3 (10.0)	8.201	0.004	0.05	5.538 (1.553-19.75)
DQB1*0303	12 (14.2)	1 (3.3)	2.625	0.105	1	4.833 (0.600-38.88)
DQB1*0401	2 (2.3)	0	0.727	0.393	1	0.000 (0.000-0.000)
DQB1*0402	30 (35.7)	0	14.54	0.00013	0.001	0.000 (0.000-0.000)
DQB1*0501	1 (1.1)	0	0.360	0.548	1	0.000 (0.000-0.000)
DQB1*0503	10 (11.9)	2 (6.6)	0.644	0.422	1	1.892 (0.39-9.177)
DQB1*0601	12 (14.2)	2 (6.6)	1.191	0.276	1	2.333 (0.490-11.1)
DQB1*0602	2 (2.3)	1 (3.3)	0.078	0.779	1	0.070 (0.061-8.094)
DQB1*0502	11 (13.0)	2 (6.6)	0.904	0.341	1	2.11 (0.439-10.12)
DQB1*0603	5 (5.9)	0	1.868	0.171	1	0.000 (0.000-0.000)
DQB1*0604	2 (2.3)	0	0.727	0.393	1	0.000 (0.000-0.000)

χ^2/Fisher's exact test. P^c, corrected *P* value after applying Bonferroni's correction; †Bold type refers to statistically significant results. HCC, hepatocellular carcinoma

with HBV infection without HCC compared with those with HCC (38.0 vs. 10.0%, χ^2=8.20, *P*=0.004, P^c=0.05, OR=5.53 and 37.5 vs. 0.0%, χ^2=14.54, *P*=0.0001, P^c=0.001, respectively) suggesting a protective effect of these alleles for the development of HCC (Table VII).

Discussion

Our results indicate that certain HLA-DQA1 and DQB1 alleles were associated with persistent HBV infection and in the development of HBV-related LC. We found that DQA1*0101/2/4, DQA1*0103 and DQB1*0302/3 showed a protective effect against the development of chronic HBV infection. These finding were consistent with reported findings in Japanese population[22] and in Korean population[12] while DQB1*0402 was associated with chronic HBV infection. Studies from south India[23] and western India[24] reported a strong association between HLA class II alleles and chronicity of HBV infection. The mechanism underlying these associations is not clearly understood. HLA class II molecules present antigen in the presence of cytokine to CD4+ lymphocytes. Among HLA class II antigens, DQ molecules are unique and many of the variable amino acid residues are located on the α-helical part of the antigen-binding site[25]. Hence, it may be hypothesized that DQA1*0101/2/4 molecule

binds and presents antigens more effectively, resulting in vigorous immune response towards HBV infection. Conversely, the association of DQB1*0402 with chronic HBV infection may be because of its inability to bind and present antigens effectively. However, further investigation needs to be done to confirm these hypotheses.

It was found that DQA1*0104, DQA1*0601, DQB1*0501, DQB1*0602 and DQB1*0604 were significantly increased in controls compared to those with persistent infection showing some protective effects from chronic HBV infection. However, these associations were not significant after Bonferroni correction. Because several HLA alleles were tested in each individual and the same data were used for comparing frequency, it was possible that one of the alleles would deviate significantly. Hence, to overcome this error, the *P* value was corrected by Bonferroni inequality method[21]. In various other studies, DQA1*0102, DQA1*0301[26,27] and DQB1*0302, DQB1*0609, DQB1*0503, DQB1*0303[12,28] alleles have been reported to be showing protective effect from developing chronic HBV infection.

Some investigators reported that HLA class II alleles were associated with development of end-stage liver disease caused by different aetiology and had a complex relationship between them[29-32]. In this study, we compared the HLA class II DQA1 and DQB1 allele frequency between patients with persistent HBV infection with and without LC. It was found that the phenotypic frequencies of DQA1*0601 and DQB1*0503 were significantly increased in HBV carriers without LC when compared with LC suggesting a protective role against the development of LC. DQB1*0601 was significantly decreased in persistent HBV infection with LC compared to those without LC, suggesting a strong protective role against the development of LC.

Some previous studies have investigated HLA class II allele association with HCC from China[11,15], Egypt[33] and Hong Kong[34] showing conflicting results. In this study, we compared the HLA class II DQA1 and DQB1 allele frequency between patients with persistent HBV infection with and without HCC and found that the phenotypic frequency of DQA1*0601 was significantly increased in persistent infection with HCC compared to without HCC suggesting a susceptible effect for the development of HCC. However, this significance was lost after Bonferroni correction. Conversely, the phenotypic frequency of DQB1*0302/3 and DQB1*0402 was significantly increased in persistent infection without HCC when compared with HCC suggesting a protective role against the development of HCC. This difference was significant even after Bonferroni correction.

In HLA association studies, once candidate genetic polymorphisms are identified in a 'derivation cohort', these findings are re-tested and confirmed in a second separate 'validation cohort'. The lack of a validation cohort was a limitation of our study.

In conclusion, three HLA-DQA1 alleles and four DQB1 alleles were found to be associated with development of chronic HBV infection, LC and HCC; DQA1*0101/2/4, DQA1*0103 and DQB1*0302/3 were associated with protection from developing chronic HBV infection, whereas DQB1*0402 was associated with susceptibility to chronic HBV infection. DQB1*0601 conferred protective effect from the development of LC, whereas DQA1*0601 showed susceptible effect for the development of HCC in patients with HBV infection. Conversely, DQB1*0302/3 and DQB1*0402 conferred protective effect from the development of HCC.

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